Biochim. Biophys. Acta 1154, 223-236; Hannun *et al., Trends Cell Biol.* 10, 73-80 (2001); Higgins *et al., Trends Biochem. Sci.* 17, 18-21 (1992)]. SPM is generally considered as the primary metabolic source of ceramide whose generation in a particular location in the cell, (e.g., the membrane) makes it suitable for mediating cellular signaling processes. An increased *de novo* synthesis of ceramide has also been described as a potential source for signaling [Bose *et al., Cell* 82, 405-414, (1995)]. Therefore, a major effort has been directed to modulate the generation of intracellular ceramide by sphingomyelinases, mostly the neutral, membrane-bound enzyme, although the acidic enzyme has also been implicated. Nevertheless, it should be emphasized that modification of the biosynthetic mechanisms such as reduction of the conversion of ceramide to SPM or glycolipids and, in parallel, its hydrolysis by ceramidases would also increase its concentration in the cell.

The role of sphingolipids in signal transduction [reviewed in L. Riboni *et al., Prog. Lipid Res.* 36, 153-195 (1997) and A. Gomez-Munoz, *Biochim. Biophys. Acta* 1391, 32-109 (1998)] have been extensively studied, and was proposed to operate through the "sphingomyelin cycle". According to this hypothesis, binding a particular extracellular ligand to its receptor activates a plasma membrane-bound sphingomyelinase, giving rise to ceramide, which acts as a mediator of the intracellular effects of the ligand. Numerous publications describe and emphasize the role of ceramide in cell killing by apoptosis as well as its effect on important cellular events such as proliferation, differentiation and reaction to stress conditions. Of particular interest are also reports that short chain, cell-permeable (e.g., C_2 or C_6) ceramides evoke biological responses that lead to cell killing. Other studies, using the precursor of ceramide, *i.e.*, sphingosine have shown its effects on cell growth and viability. Furthermore, sphingosine was shown to inhibit protein kinase C and increase the intracellular concentration of calcium ions. The phosphorylated form of sphingosine, *i.e.*, sphingosine-1-phosphate has been shown to be a potent activator of phospholipase D. And di- or tri- methylated

converting it to glucosylceramide, making them resistant to a series of chemotherapeutic drugs.

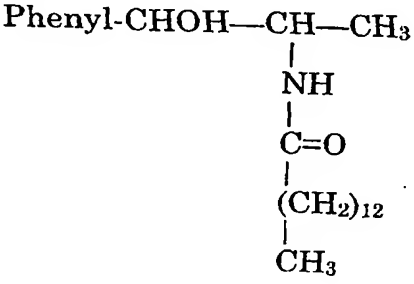
Of special interest is the mechanism proposed for the anticancer drug hexadecylphosphocholine [HePC, Wieder *et al., J. Biol. Chem.* 273, 11025-11031, (1998)]. This is an antiproliferative drug, which is currently used for the treatment of extraneous metastases of mammary carcinoma and has been shown to induce apoptosis at a concentration of 25 μM. The above publication provides support that HePC, which inhibits the biosynthesis of phosphatidylcholine exerts a secondary effect by decreasing the biosynthesis of sphingomyelin and consequently increasing the levels of ceramide and it is probably the latter that is responsible for the proapoptotic properties of HePC. And, indeed the authors showed that the PC-induced apoptosis was blocked by Fumonisin B1, an inhibitor of ceramide synthesis. And, short-chain, membrane-permeable ceramides additively increased the apoptotic effect of HePC.

Another major aspect of the metabolism of the sphingolipids is their accumulation in organs of patients afflicted with the genetic lipid storage diseases, such as Gaucher disease (β-glucosidase), Tay-Sachs disease (β-*N*-acetyl hexosaminidase); Niemann-Pick disease (acid sphingomyelinase), Krabbe disease (β-galactosidase), Metachromatic leukodystrophy (arylsulfatase A), Fabry disease (ceramidase) and Farber disease (α-galactosidase). Each of these diseases is due to a mutation in a gene encoding a lysosomal sphingolipid hydrolase (shown in brackets). Consequently, the activity of the respective hydrolase is considerably reduced resulting in accumulation of the respective sphingolipid in the patients' organs.

Being a metabolic disorder, the metabolic defect and accumulation of the corresponding sphingolipid is a life-long phenomenon. Three forms of therapy are being used or considered. 1. Enzyme replacement therapy, in

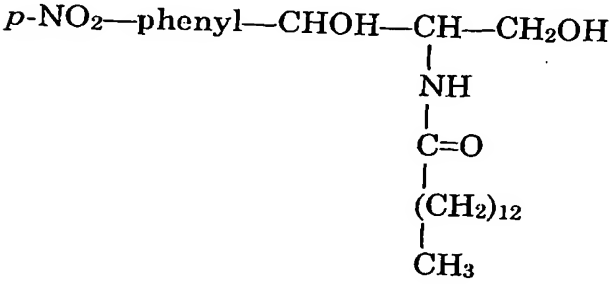
has been shown to be an inhibitor of glucosphingolipid [Vunnam & Radin *Chem. Phys. Lipid* 26, 265 (1980)].

Acyl phenyl amino alcohol (MAPP):



has been shown to inhibit ceramidase, resulting in an inhibition of cell growth [Bielawska *et al.*, *J. Biol. Chem.* 271, 12646-12654 (1996)].

Esters of *p*-nitrophenyl-amino-propanediol:



have been shown to inhibit cell differentiation. [Bielawska *et al.*, *J. Biol. Chem.* 267, 18493-18497 (1992)].

Other non-natural derivatives of sphinglipids affect cell growth and differentiation. For example N,N,N-trimethyl sphingosine has been shown to inhibit cell growth [Endo *et al.*, *Cancer Research*, 51, 1613-1618 (1991)]. C_8 ceramide in which the amide group was replaced by $—NH—(CH_2)_7CH_3$: $CH_3(CH_2)_{12}—CH=CH—CHOH—CHNH[(CH_2)_7CH_3]—CH_2OH$ induced apoptosis [Karasavvas *et al.*, *Eur. J. Biochem.* 236, 729-731 (1996)].

X represents hydrogen or the group $—OR_4$ in which R_4 is a linear or branched, saturated or unsaturated C_1-C_6 alkyl or alkenyl chain which may be optionally substituted with hydroxy;

Y represents $—NH_2$, NHR^x wherein R^x is hydrogen, a linear or branched alkyl or alkenyl chain which may be optionally substituted with hydroxy, an amino protecting group, $—NH—\overset{\overset{S}{\|}}{C}—R_1$, $—NH—\overset{\overset{S}{\|}}{C}—NH—R_1$, $—NH(SO_2)R_1$, $—NR_1R_2$, $—N^+R_1R_2R_3$, wherein R_1, R_2 and R_3, which may be identical or different each represent $C_{1\text{-}6}$alkyl or $C_{1\text{-}6}$alkenyl, a group $—NH—\overset{\overset{O}{\|}}{C}—(CH_2)_n$—adamantane wherein n is zero or an integer of from 1 to 20, a group —NH—adamantane, a group $—NH—\overset{\overset{O}{\|}}{C}—(CH_2)_n\overset{\overset{O}{\|}}{C}—NH—$ polymer, where "polymer" designates a natural or synthetic biocompatible polymer having a molecular weight between 10^3 and 10^6 daltons;

Z represents hydrogen, —OH, a mono- or disaccharide, a monosaccharide sulfate and choline phosphate;

with the proviso that

- Y cannot represent NH_2 when R represents an alkyl, the group $CH_3(CH_2)_mCH = CH—$, phenyl or nitro phenyl; and

Y cannot represent the groups $—NR_1R_2$ or $—N^+R_1R_2R_3$, or NHR_4 where R_4 represents octyl when R_1 represents a methyl, R represents the group $CH_3(CH_2)_mCH = CH—$ and Z represents —OH;

and isomers and pharmaceutically acceptable salts thereof.

The invention also relates to a pharmaceutical composition comprising as active ingredient a compound of formula (I) wherein the substituents are as

Abbreviations: Via (viable), C (cells), Contr (control), Inhi (inhibitor), Conc (concentration).

Figure 2 – *AD-2646 cytotoxic effect on TSU-PR1cells*
The cytotoxic effect of AD-2646 was examined using increasing concentrations of AD-2646 for two days. The total protein content was measured. Abbreviations: Prot (protein).

Figure 3 – *Effect of AD-2646 on sphingolipids metabolism (SPM)*
HL60 cells were incubated with increasing concentrations of AD-2646 for 3 hours in the presence of 2.5 μM Bodipy-C3-ceramide. After extraction, the lipids were applied onto a thin layer chromatography plate, and the fluorescence of Bodipy-C3 - sphingomyelin (SPM) was quantified.

Figure 4 – *Effect of AD-2646 on sphingolipids metabolism (GC)*
HL60 cells were incubated with increasing concentrations of AD-2646 for 3 hours in the presence of 2.5 μM Bodipy-C3-ceramide. After extraction, the lipids were applied on a thin layer chromatography plate, and the fluorescence of Bodipy-C3-cerebroside (GC) was quantified.

Figure 5 – *AD-2646 inhibits SPM synthesis in TSU-PR1 cells*
TSU-PR1 cells were incubated for 3 hours in the presence of increasing concentrations of AD-2646 and the fluorescence of Bodipy-C3 - sphingomyelin (SPM) was quantified.

Figure 6 – *Inhibition of sphingolipids metabolism by the compounds of the invention*
HL60 cells were incubated with the different compounds AD-2672, AD-2673 and AD-2674, each at 5 and 10 μM. The effect of the different compounds on synthesis of Bod3-SPM and Bod3-GC was examined. The relative quantity

hydroxy, an amino protecting group, —NH—C(=S)—R_1, —NH—C(=S)—NH—R_1, —NH(SO_2)R_1, —NR_1R_2, —$N^+R_1R_2R_3$, wherein R_1, R_2 and R_3, which may be identical or different each represent C_{1-6}alkyl or C_{1-6}alkenyl, a group —NH—C(=O)—$(CH_2)_n$—adamantane wherein n is zero or an integer of from 1 to 20, a group —NH—adamantane, a group —NH—C(=O)—$(CH_2)_n$C(=O)—NH—polymer, where "polymer" designates a natural or synthetic biocompatible polymer having a molecular weight between 10^3 and 10^6 daltons;

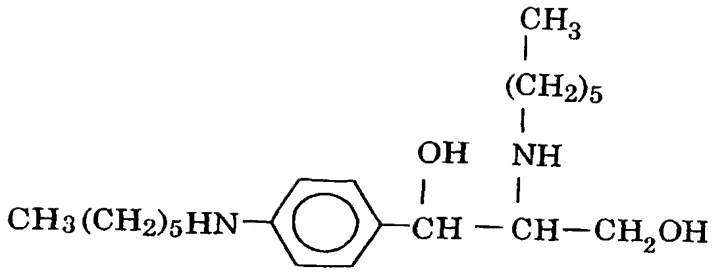
Z represents hydrogen, —OH, a mono- or disaccharide, a monosaccharide sulfate and choline phosphate;
with the proviso that

- Y cannot represent NH_2 when R represents an alkyl or alkenyl chain, the group $CH_3(CH_2)_mCH = CH$—, phenyl or nitro phenyl; and
- Y cannot represent the groups —NR_1R_2 or —$N^+R_1R_2R_3$, or NHR4 where R_4 represents octyl when R_1 represents methyl, R represents the group $CH_3(CH_2)_mCH = CH$— and Z represents —OH;

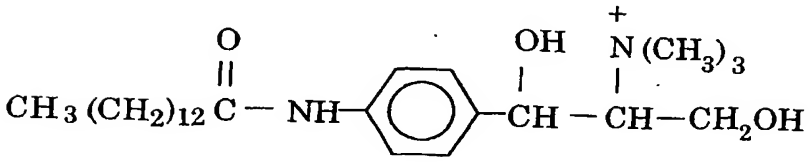
and isomers and pharmaceutically acceptable salts thereof.

Preferred compounds of formula (I) are those in which R designates aminophenyl or nitrophenyl.

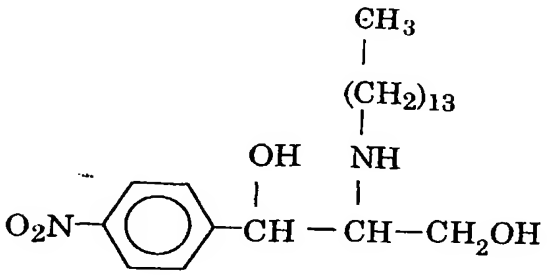
Also preferred are compounds of formula (I) in which Y represents —NH_2 or —NHR^x, particularly compounds in which R^x designates an alkyl chain.



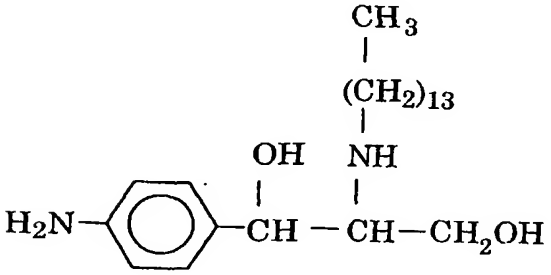
(designated AD-2665)



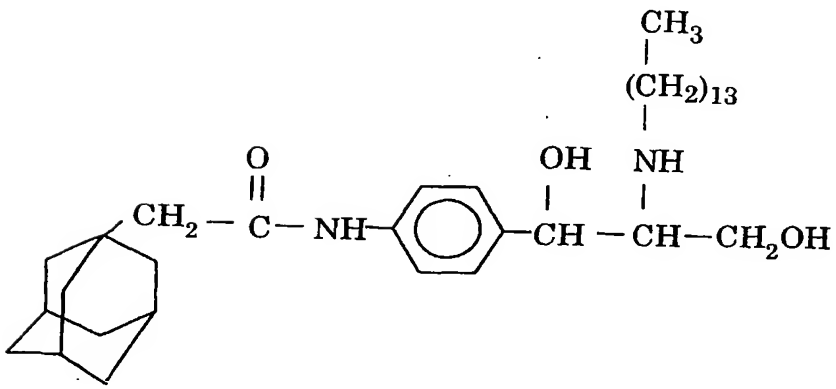
(designated AD-2687)



(designated AD-2646)



(designated AD-2672)



(designated AD-2754)

Still further, the compounds of the invention may be used in the treatment of parasitic diseases such as malaria and leishmania. Pharmaceutical compositions for the treatment of such parasitic diseases are also within scope of the present invention.

The compounds of the invention are generally provided in the form of pharmaceutical compositions. Said compositions are for use by injection or by oral uptake.

The pharmaceutical compositions of the invention generally comprise a buffering agent, an agent which adjusts the osmolarity thereof, and optionally, one or more carriers, excipients and/or additives as known in the art, e.g., for the purposes of adding flavors, colors, lubrication, or the like to the pharmaceutical composition.

Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the subject to be treated. While formulations include those suitable for rectal, nasal, preferred formulations are intended for oral or parenteral administration, including intramuscular, intradermal, subcutaneous and specifically intravenous administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy.

Carriers may include starch and derivatives thereof, cellulose and derivatives thereof, e.g., microcrystalline cellulose, xantham gum, and the like. Lubricants may include hydrogenated castor oil and the like.

A preferred buffering agent is phosphate-buffered saline solution (PBS), which solution is also adjusted for osmolarity.

it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above.

In the case of sterile powders for the preparation of the sterile injectable solutions, the preferred method of preparation are vacuum-drying and freeze drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For oral administration, the composition of the invention may be mixed with nutritive feed material or water supplies for the subject to be treated. It is contemplated however that the effective composition can either be mixed with the nutritive feed material or water or fed to the subject separately.

The preparation of pharmaceutical compositions is well known in the art and has been described in many articles and textbooks, see e.g., Remington's Pharmaceutical Sciences, Gennaro A.R. ed., Mack Publishing Company, Easton, Pennsylvania, 1990, and especially pages 1521-1712 therein.

Additives may also be designed to enhance uptake of the active agent across cell membranes. Such agents are generally agents that will enhance cellular

well known to the person of skill in the art. See e.g., Current Protocols in Immunology, Coligan *et al.* (eds), John Wiley & Sons. Inc., New York, NY.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise.

Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

The following examples are thus only representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications can be made without departing from the spirit and intended scope of the invention.

dichloromethane containing increased amounts of methanol. Yield: 1.2 gr; M.S. $I^+ = 296$.

2. *Preparation of (2S,3R) 2-amino-1-(4-aminophenyl)-1,3-propanediol (AD-2516)*

5 gr (2S,3R)-2-amino-1-(4-aminophenyl)-1,3-propanediol were dissolved in 150 ml methanol, 100 mg 10% Pd/C were added and the solution was hydrogenated for 6 h at 50 Psi in a hydrogenator at room temperature. The catalyst was removed by filtration and the solution was evaporated to dryness. The dry compound was used for synthesis without further purification. Yield: 5 gr; M.S. $I^+ = 183$.

3. *Preparation of (2S,3S)-2-(N-octylamine)-1-(4-N-octylaminophenyl)-1,3-propanediol (AD-2670)*

3 gr (2S,3S)-2-amino-1-(4-aminophenyl)-1,3-propanediol (prepared as in AD-2516) were dissolved in 100 ml methanol:water 1:1 in a round bottom flask. The solution was magnetically stirred and 1 ml octyl aldehyde (octanal) was added followed by 1 ml acetic acid. The solution was stirred for 15 min and 1 gr of sodium cyanoborohydride $NaCNBH_3$ was added in portions during 2 hours. The solution was stirred for another 3 h, then transferred to a separatory funnel and 100 ml water, and 50 ml dichloromethane and 25 ml methanol were added. Following shaking, the lower phase was collected and the upper, aqueous-methanolic phase was extracted twice with 50 ml CH_2Cl_2:MeOH, 3:1. The combined organic phases were washed with 100 ml H_2O, dried for 2 hours over $MgSO_4$, filtered and the solution was evaporated to dryness. The residue was dissolved in minimal amount of dichloromethane methanol, 1:1, and loaded onto a 2 × 50 cm silica gel. The column was eluted with increasing amounts of methanol in dichloromethane. Yield: 2.1 gr; M.S. $I^+ = 407$.

6. *Preparation of (2R,3R)-2-amino-1-(4-N-butyroylaminophenyl)-1,3-propanediol (AD-2602)*

2 gr of (2R,3R)-2-BOC-amino-1-(4-*N*-butyroylaminophenyl)-1,3–propanediol (prepared as in the preparation of compound AD-2522, using butyric acid instead of hexadecanoic acid), were dissolved in 10 ml trifluoroacetic acid (TFA):dichloromethane, 1:1. The mixture was transferred to a 20 ml screw-capped test tube and kept for 2 h at 37°C with occasional stirring, then evaporated to dryness in a ratavapor and the residue purified using a silica gel column eluted with dichloromethane and increasing amounts of methanol. Yield: 1.2 gr; M.S. $I^+ = 353$.

7. *Preparation of 2-N,N,N-trimethylamino-1-(4-N-dodecanoyl-aminophenyl)-3-propanol (AD-2687)*

2 gr of 2-amino-1-(4-*N*-dodecanoylamino phenyl)-3-propanol was prepared as in the preparation of AD-2602 (6) from the corresponding starting material. The compound was dissolved in 20 ml of methanol in a screw-capped pressure glass tube. 4 ml CH_3I were added followed by 1 gr of sodium carbonate. The pressure glass tube was sealed and immersed in a heating bath at 80°C for 12 h. The tube was cooled and opened. The solution was transferred to a round bottom flask and evaporated to dryness. The residue was dissolved in a minimal amount of dichloromethane:methanol, 1:1, loaded onto a silica gel column and eluted with solutions of dichloromethane and increasing methanol. Yield: 1.6 gr; M.S. $I^+ = 408$.

8. *Preparation of sphingosyl-N-butyl sulfonamide (AD-2208-B)*

200 mg of sphingosine were dissolved in 10 ml dichloromethane:methanol, 2:1, in a 25 ml Erlenmeyer flask. 200 µl of butylsulfonylchloride were added and the solution was stirred for 10 min. 300 µl of triethylamine were then added in 50 µl portions during 1 h and the solution was stirred overnight. The solution was transferred to a 250 ml separatory funnel, a mixture of 50 ml dichloromethane, 15 ml methanol and 25 ml water were added and

methanol, 1:1, loaded onto a small silica gel column and the product eluted with increasing ratios of methanol and dichloromethane. Yield: 70 mg; M.S. $I^+ = 468$.

11. *L-erythro sphingosyl phosphorylcholine-N-hexyl* ***(AD-2144)***

100 mg of sphingosyl phosphoryl choline were dissolved in 25 ml methanol:water 1:1 in 100 ml Erlenmeyer flask. The solution was stirred on a magentic stirrer and 100 mg hexanal and 250 µl acetic acid were added. The mixture was stirred for 20 min and 100 mg $NaCNBH_3$ was added. After overnight stirring at room temperature the solvents were evaporated to dryness and the residue was washed and purified as was done in preparation of AD-2209 (9). Yield: 65 mg; M.S. $Na^+ = 562$.

12. *D,L-1,3-dihydroxy-2-[amino(N-FMOCpropyl-3-amine)]-octadecane* ***(AD-2751)***

300 mg of DL-1,3-dihydroxy-2-aminooctadecane were dissolved in 50 ml methanol:water 1:1. 100 mg FMOC β-alaninal (*N*-FMOC 3 aminopropanal) were added followed by 0.5 ml acetic acid. The solution was stirred during 20 min and 200 mg $NaCNBH_3$ were added in portions during 1 hour. The mixture was left to stir for 5 hours, evaporated to dryness, redissolved in a minimal amount of dichloromethane: methanol, 2:1, and purified by column chromatography on a silica gel column and with increasing ratios of methanol and dichloromethane. Yield: 200 mg; M.S. $I^+ = 581$.

13. *D,L-1,3-dihydroxy-2-[amino(3-aminopropyl)]-octadecane* ***(AD-2752)***

50 mg D,L-1,3-dihydroxy-2-[amino(*N*-FMOC propyl-3-amine)] octadecane were dissolved in 4 ml methanol and 1 ml piperidine. The mixture was stirred for 30 min, then evaporated to dryness under a nitrogen stream. The product was purified using a preparative thin layer chromatography silica plate developed with a solution of dichloromethane:methanol:ammonium: hydroxide:water, 80:20:1:1. The product (viewed with a UV-lamp) was

250 ml round bottom flask equipped with a reflux condenser and stirred on a magnetic stirrer. The solution was evaporated to dryness and dissolved in 200 ml dichloromethane methanol, 2:1, transferred to a 500 ml separatory funnel and washed with 75 ml 0.2N HCl. Phases were separated and the organic phase was washed again with 75 ml 0.1N HCl and 15 ml methanol. The organic phase was separated and dried on 5 gr magnesium sulfate, filtered and evaporated to dryness. The resulting oil was dissolved in minimal amount of warm ether and left to crystallize overnight at –20°C. Crystals were filtered at low temperature and recrystallized from hot ether containing 3% of H_2O. Yield, 3.6 gr. This compound was quantified in a spectrophotometer providing a peak at 270 nm. Its molar extinction coefficient was 7.84 optical density units per micromole per ml. M.S. I^+ = 409.

NMR: ($CDCl_3$) 0.88, t(3H); 1.26, m (22H); 1.48, m (2H); 2.54, m (1H); 2.73, m (2H);

3.30, broad s (3H); 7.6, d (2H); 8.2, d (2H).

18. *(2R,3R)-2-(N-tetradecylamine)-1-(4-aminophenyl)-1,3-propanediol (AD-2672)*

2 gr of (2R,3R)-2-(*N*-tetradecyl amine)-1-(4-nitrophenyl)-1,3- propanediol (AD- 2644) were hydrogenated as was done in the preparation of AD-2516 (2). The resulting oil was purified by column chromatography on silica gel eluted with increasing concentrations of methanol in dichloromethane. Yield: 1.5 gr; M.S. I^+ = 378.

19. *(2R, 3R)-2 amino-1-(N-dodecanoylaminophenyl)-1,3-propanediol (AD-2673)*

This compound was prepared from 2 gr of (2R,3R)-2(*N*-tBOC amino)-1-(*N*-dodecanoylaminophenyl)-1,3-propanediol (AD-2582) using the same protocol as in the preparation of AD-2602 (6). Overall Yield: 1.3 gr M.S. I^+ = 365.

phenyl)-1,3-propanediol (AD-2646) in 6- or 24-well dishes. The compound was added as solutions in dimethylsulfoxide (DMSO), ensuring that the concentration of this solvent did not exceed 0.1% of the volume of the culture medium. After 2 days the cells were collected, washed twice with saline, treated with trypan blue and the number of living (non-blue) cells was counted. Fig. 1 indicates an IC50 value of 5 μM.

Kinetic of the observed killing effect of the AD-2646 compound was next examined. HL60 cells were incubated with 40 μM of AD-2646 for 1, 3, 5 and 7 hours. Already after 1 hour a reduction of 60% of the viable cell number was observed.

The effect of the AD-2646 compound on cell viability was further supported when the total protein content was quantified. HL60 cells were incubated as described above, with different concentrations of AD-2646. After 2 days the cells were collected, washed twice with saline, dispersed and following a short pulsing with a probe-sonicator their protein content was quantified by the Bradford procedure. Similarly to the viable cell counting results, the protein measurements indicated an IC50 value of 5 μM.

To analyze whether the AD-2646 compound may mediate its effect on cell viability and on total protein content, in cooperation with other compounds, the cooperative effect of this compound and Taxol was next evaluated. HL60 cells were incubated for two days with 4 μM AD2646 in the presence or absence of 2 ng Taxol. As shown in Table 1, cooperation of both compounds caused significant decrease in the total protein quantity indicating a synergism between the two respective compounds.

Table 2

Effect of different compounds on cell viability

Compound	Cell line	IC50
AD-2673	HL60	7.5μM
AD-2620	HL60	3μM
AD-2687	HL60	2μM
AD-2665	MCF7-AdrR	7μM
AD-2665	MCF7-NCi	3μM

Example 4

The apoptotic effect of different compounds

In order to examine whether the observed effect on cell viability involves induction of apoptosis, the apoptotic effect of several compounds of the invention was next examined.

Myeloid, leukemic U937 cells were incubated for 24 hours in the absence or in the presence of 5 μM AD-2672 and AD-2665. Cells were then collected and the percent of apoptotic cells was determined using a kit quantifying the DEVDase, caspase 3 activity. The number of apoptotic cells incubated with AD-2672 exceeded 6-fold those in the control cells. Cells incubated with AD-2665 exceeded 2.8- fold those in the control cells.

The apoptotic effect of three different compounds was further examined on HL60 cells using a flow cytometry method. Cells were incubated with AD-2646, AD-2665 or AD-2687 at 3 and 24hr and increasing compound concentrations, collected and washed. Washed cells were then treated with 5% Triton and stained with propidium iodide 0.5% mg in 0.1% sodium citrate pH 7.4. Analysis was performed using a Becton Dickinson Fluorescence Activated Cell Sorter (FACS).

Cells were next extracted with chloroform- methanol containing 2% acetic acid 1:1 (by volume) and the medium was shaken with an equal volume of chloroform-methanol, 1:1 (by volume). The phases were separated by centrifugation and the lower chloroform phase was collected. The solvents were evaporated and applied to thin layer chromatography silica gel plates (Whatman 4865-821) with a concentrating zone.

Plates were developed as follows: For medium: in chloroform-methanol-H_2O, 75:25:4 by volume. For cells: plates were first developed with chloroform-methanol, 9:1, then dried and re-run in chloroform-methanol: H_2O, 65:35:4.

Standards of Bodipy-C3-ceramide: Bodipy-C3-glucosylceramide (Bod3-GC) and Bodipy C3 sphingomyelin (Bod3-SPM) were used as markers. The fluorescence of the respective Bod3-SPM and Bod3-GC spots was quantified using a Fuji FLA-2000 scanner.

Generally, results indicated that Bod3-SPM was present both in the cells and medium, whereas Bod3-GC was present practically only in the cells.

As shown in Table 3, incubation of HL60 cells with different concentrations of AD-2646 resulted in reduction of the Bod3-SPM (cell + medium), with IC50 values of about 6 μM (Fig. 3), while the IC50 for Bod3-GC reduction was about 12 μM (Fig. 4).

The effect of AD-2646 on sphingolipid metabolism was further examined using TSU-PR1 prostate cancer cells. Results showed reduction of Bod3-SPM had IC50 values of about 5 μM (Fig. 5).

Bod-C3-GC secreted into the drug-resistant cell medium were higher by 5-9-fold of those secreted by their drug-sensitive counterparts.

Example 6

Inhibitory effect of AD-2144 on sphingomyelinases

N-hexyl-sphingosyl phosphorylcholine (AD-2144) was tested as to its inhibitory effect on sphingomyelinases, acidic (*i.e.*, with an optimum at about pH 5) or neutral (with a pH optimum at about pH 7.4). For this purpose a sonicate of HL60 leukemic cells was used as enzyme source. Increasing concentrations of AD-2144 were dispersed by a mixture of fluorescent and non-fluorescent sphingomyelin (Bodipy-C12-SPM:SPM, 1:19), buffer and 0.25% Triton-X100 in a volume of 100 µl. For acid sphingomyelinase 0.4M acetate buffer pH 5.0 was used; for neutral sphingomyelinase 0.2M Tris buffer, pH 7.4 and 5 mM magnesium chloride. To 100 µl of these dispersions, 100 µl of HL60 cells sonicate were added. Incubation was 2-3 hours after which 0.8 ml of chloroform:methanol 2:1 were added, stirred and the lower, chloroform phase was collected, dried and applied to a thin layer chromatography plate. The plate was developed in a mixture of chloroform and methanol, 87:3. The fluorescence of the product, *i.e.*, Bodipy-C12-ceramide, was quantified.

For acid sphingomyelinase as well as for neutral spingomyelinase: at 300 µM of AD-2144 there was a reduction of over 60% in Bodipy-C12-ceramide. Table 4 shows reduction of the Bodipy-C12-ceramide by the AD-2144 compound.

Table 5

AD-2646 viability test

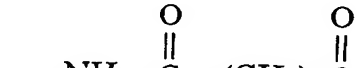
AD-2646 Concentration	No. of mice in each day					
	1	2	3	4	5	6
0	5	5	5	5	5	5
1mg/kg	5	5	5	5	5	5
5mg/kg	5	5	5	5	5	5
10mg/kg	5	5	5	5	5	5
25mg/kg	5	5	5	5	5	5
50mg/kg	5	5	4	3	2	1

Example 8

2×10^5 *Leishmania major promastigote* cell, each in a volume of 100 µl were added to 100 µl medium (RPMI 1640 culture medium complemented with 20% FCS, 1% penicillin and 1% streptomycin) in 96-well plates containing zero and increasing concentrations of (2S,3R)-2-*N*-aminohexyl-1-(4-*N*-hexylaminophenyl)-1,3-propanediol (AD-2663) in 2-fold serial dilution. After incubation period (3 h, 27°C) the number of cells was determined by counting on aliquot from each well on a Neulander cell counter under a microscope. The IC50 for cell reduction was 7 µM.

represent C_{1-6}alkyl or C_{1-6}alkenyl, a group

—NH—C—$(CH_2)_n$—adamantane wherein n is zero or an integer of from 1 to 20, a group —NH—adamantane, a group



—NH—C—$(CH_2)_n$C—NH—polymer, where "polymer" designates a natural or synthetic biocompatible polymer having a molecular weight between 10^3 and 10^6 daltons;

Z represents hydrogen, —OH, a mono- or disaccharide, a monosaccharide sulfate and choline phosphate;

with the proviso that

- Y cannot represent NH_2 when R represents an alkyl, the group $CH_3(CH_2)_mCH = CH$—, phenyl or nitro phenyl; and

Y cannot represent the groups —NR_1R_2 or —$N^+R_1R_2R_3$, or NHR_4 where R_4 represents octyl when R_1 represents a methyl, R represents the group $CH_3(CH_2)_mCH = CH$— and Z represents —OH;

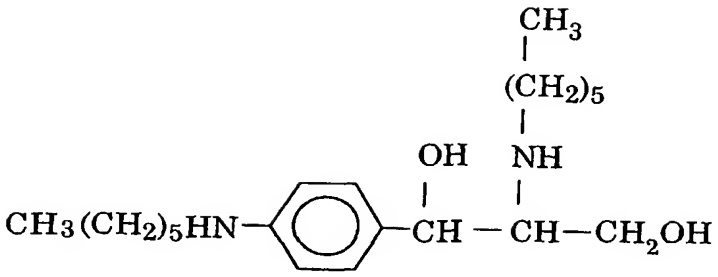
and isomers and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein Y is —NH_2 or NHR^x.

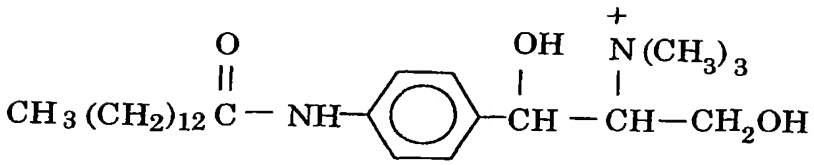
3. A compound according to claim 1 or claim 2, wherein R is nitrophenyl.

4. A compound according to any one of claims 1 or 2, wherein R is aminophenyl or alkylaminophenyl.

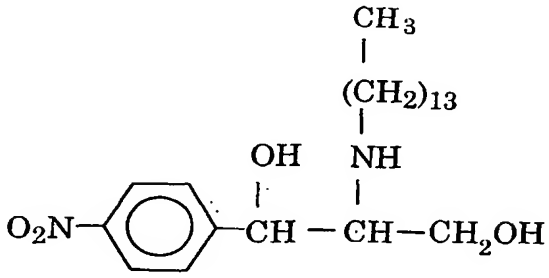
5. A compound according to any one of claims 1 to 4, wherein Z is —OH.



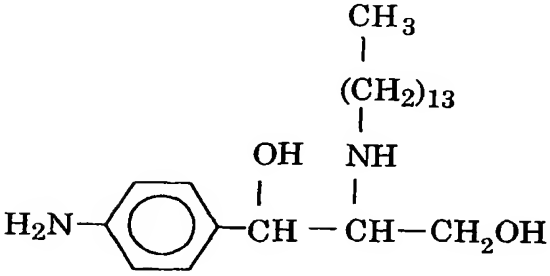
(designated AD-2665)



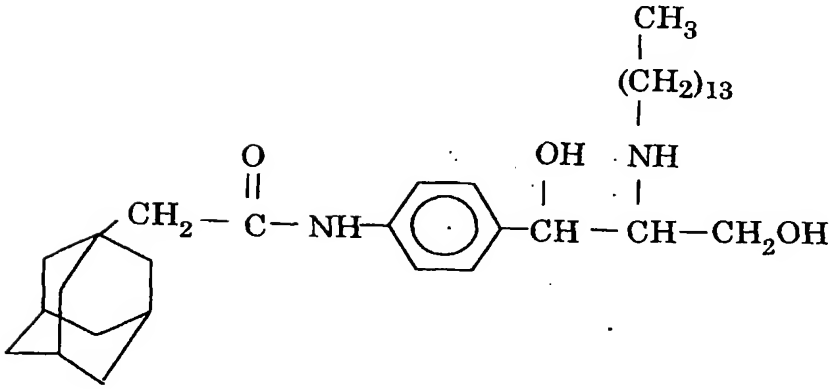
(designated AD-2687)



(designated AD-2646)



(designated AD-2672)



(designated AD-2754)

12. Use of a compound of formula (I) wherein the substituents are as defined in claim 1 as an inhibitor of any one of acidic, neutral and alkaline sphingomyelinases, acidic, neutral and alkaline ceramidases, α-galactosyl synthetase, ceramide synthetase, sphingomyelin synthetase and glycoceramides synthetase.

13. A pharmaceutical composition according to claim 8, for the treatment of cancerous diseases.

14. Use of a compound of formula (I) wherein the substituents are as defined in claim 1, for killing of wild type and drug-resistant cancer cells.

15. Use according to claim 14, for the selective killing of drug-resistant cancer cells.

16. A pharmaceutical composition according to claim 8, for the treatment of parasitic, viral, bacterial, fungal and prion diseases.

17. A pharmaceutical composition according to claim 16, wherein said parasitic disease is malaria or leishmania.

18. Use of a compound of formula (I) wherein the substituents are as defined in claim 1, as an antimalarial or antileishmanial agent.

19. A method of treating a lipid storage disease in a patient in need of such treatment comprising administering to said patient a therapeutically effective amount of a compound of formula (I) wherein the substituents are as defined in claim 1 or of pharmaceutical composition comprising the same.

20. A method according to claim 19, wherein said lipid storage disease is selected from Gaucher disease, Tay-Sachs disease, Niemann-Pick disease,